Growth rate and productivity of a microbial mutualism depends on the *Desulfovibrio* genotype

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ESPP2 Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT — OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

VIMSS Virtual Institute for Microbial Stress and Survival — MIT Massachusetts Institute of Technology — Missouri — University of Washington — Montana State University — Southern Illinois University Carbondale — Oklahoma — Sandia National Laboratories — Oak Ridge National Laboratory — Berkeley — http://vimss.lbl.gov/

INTRODUCTION

Desulfovibrio have metabolic capabilities that enable them to grow on diverse combinations of electron donors and acceptors. Genetic differences among strains of *Desulfovibrio* likely affect their competitive ability across resources. For example, some strains may be more competitive growing with lactate and sulfate while others may have adaptations allowing superior syntrophic growth in cooperation with hydrogen consuming species in the absence of sulfate. If we develop a working knowledge of the relationships between competitive ability for each resource and the physiological and genetic differences between strains that enhance performance, then we could eventually predict which *Desulfovibrio* strains and alleles will proliferate in a particular environment. This predictive ability is of particular interest to bioremediation and environmental process control (DOE priority areas).

Here, we examine competitive differences between *Desulfovibrio* strains growing syntrophically with the hydrogenotrophic methanogen, *Methanococcus maripaludis*. They gain energy from the following reactions:

Desulfovibrio partial reaction:
$2\text{Lactate} + 4H_2O \rightarrow 2\ \text{acetate} + 2H^+ + 2HCO_3^- + 4H_2$

Methanococcus partial reaction:
$4H_2 + 2H^+ + 2HCO_3^- \rightarrow CH_4 + 3H_2O$

Combined reaction:
$2\text{Lactate} + H_2O \rightarrow 2\ \text{acetate} + CH_4 + H^+ + HCO_3^-$ $\Delta G = -144$ kJ

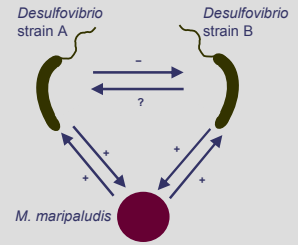
Specifically, we asked:

1. How variable is syntrophic growth rate and efficiency among *Desulfovibrio*?
2. Are strains that cause superior syntrophic growth also competitively dominant?
3. What characteristics are associated with faster, more efficient syntrophic growth?

RESULTS: COMPETITION BETWEEN *DESULFOVIBRIO* STRAINS IN SYNTROPHY

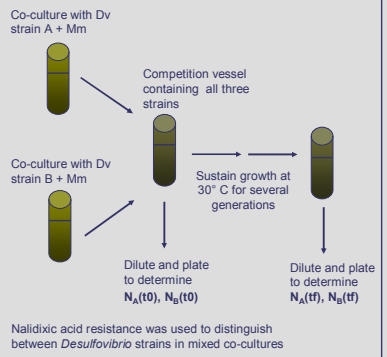
Putative interactions in competition

In nature, where multiple strains of *Desulfovibrio* may exist in the same community, syntrophic interactions are more likely to resemble the diagram below than a pure culture. We might assume that *Desulfovibrio* that grow fast syntrophically are most likely to predominate, but *Desulfovibrio* could hypothetically interact in unexpected ways.



In addition to intraspecific competition, *Desulfovibrio* could affect each other by:
- Production of toxins or antagonists
- Stimulation of methanogen growth
- Changing the hydrogen concentration

Competition experimental design

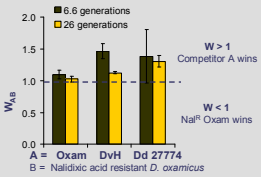


Nalidixic acid resistance was used to distinguish between *Desulfovibrio* strains in mixed co-cultures

Fitness Calculation:

$$W_{AB} = \frac{\ln[\,N_A(tf) / N_A(t0)\,]}{\ln[\,N_B(tf) / N_B(t0)\,]}$$

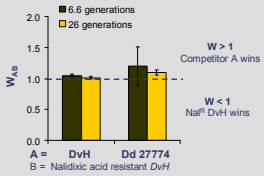
Competitions with *D. oxamicus*



Key to abbreviations
DvH = *D. vulgaris* Hildenborough
Oxam = *D. oxamicus*
Dd 27774 = *D. desulfuricans* 27774

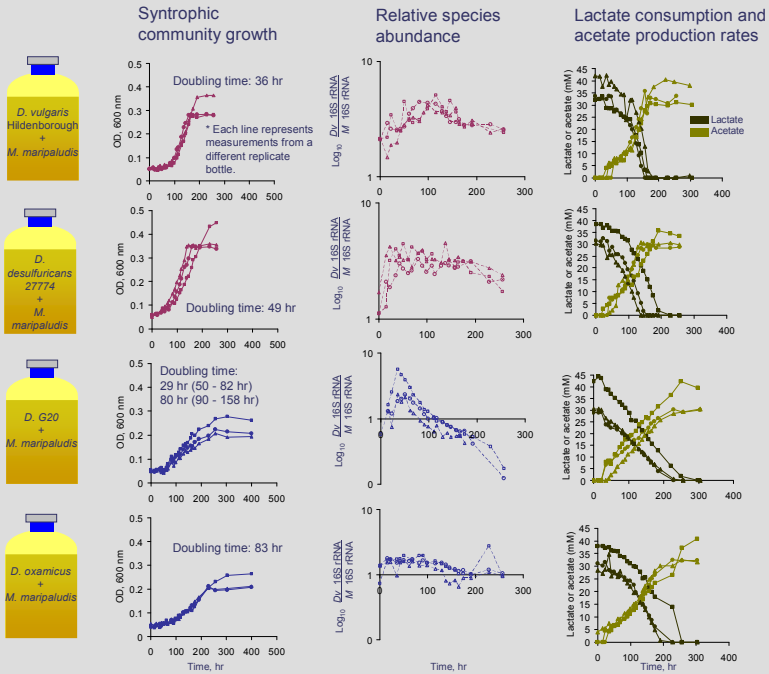
** Points indicate the mean of three independent competitions. Error bars show the standard deviation.

Competitions with *D. vulgaris* Hildenborough



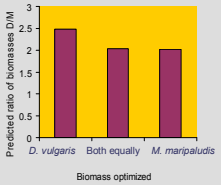
- Both faster growing syntrophs had a greater competitive advantage against the slower growing syntroph, *D. oxamicus*.
- This effect could not be attributed to the Nalidixic acid resistance marker alone (leftmost competitions)
- Fitness differences were greater and more variable after only 6.6 generations of competition than after 26 generations
- Even though DvH syntrophic growth was faster than Dd 27774 syntrophic growth, Dd 27774 was a slightly better competitor when both strains were combined with *M. maripaludis*.
- This effect could not be attributed to the Nalidixic acid resistance marker alone (leftmost competitions)

RESULTS: CHARACTERISTICS OF FAST AND HIGH YIELD SYNTROPHIC GROWTH



Relationship between co-culture growth rate, species dominance, and flux-balance modeling

- In both faster growing co-cultures (*D. desulfuricans* 27774 +Mm, and *D. vulgaris* Hildenborough +Mm), *Desulfovibrio* dominated over *Methanococcus* between 2 and 4-fold throughout the course of batch culture growth
- In slower growing co-cultures, *Methanococcus* dominated over *Desulfovibrio* (*D.* G20 +Mm) or both species had similar abundance (*D. oxamicus* + Mm)
- These results agree with predictions of a flux balance model of the co-culture, published previously**. The model predicts that *Desulfovibrio* will predominate at least 2-fold in a syntrophic community that is optimized for growth of either or both species.



**Stolyar S, S Van Dien, KL Hillesland, N Pinel, TJ Lie, JA Leigh, and DA Stahl. (2007) Metabolic modeling of a mutualistic microbial community. Mol. Syst. Biol. 3:92

Co-culture growth and dynamics of lactate metabolism

- As would be expected, lactate consumption rates were similar to growth rates. Faster growing co-cultures consumed lactate and produced acetate faster than slower growing co-cultures.
- In three of the co-cultures, lactate was consumed very slowly in the early stage of co-culture growth, and more rapidly later on. The co-culture with *D.* G20, however maintained a constant, linear rate of lactate consumption and acetate production over time. This result, and the dominance of *Methanococcus* in this co-culture, may indicate a tighter coupling between the species.

CONCLUSIONS

1. How variable is syntrophic growth rate and efficiency among *Desulfovibrio*? Syntrophic growth by *Desulfovibrio* strains paired with the same *Methanococcus* strain can vary considerably. In the work presented here, doubling times for co-cultures could be as low as 36 to as high as 83 hrs and the biomass achieved on the same resource concentration varied by ~30%.

2. Are strains that cause superior syntrophic growth also competitively dominant? Both strains capable of fostering more rapid syntrophic growth had a competitive advantage against *D. oxamicus* in syntrophic growth conditions. However, when the difference between competitors in co-culture growth was less pronounced, the more slowly growing strain (*D. desulfuricans* 27774) had a slight advantage over *D. vulgaris* Hildenborough in direct competition. Thus, growth rate of co-cultures may be an indicator of competitive fitness, but other factors cannot be ruled out.

3. What characteristics are associated with faster, more efficient syntrophic growth? The rate of co-culture growth is associated with the relative dominance of *Desulfovibrio* vs *Methanococcus* in co-culture. *Desulfovibrio* dominated in the faster growing co-cultures. This suggests a relationship between the rate of utilization of lactate via syntrophic growth and the manner in which the energy from lactate is partitioned between syntrophic partners. For *Methanococcus*, improved yield may come at the cost of slower growth.

ACKNOWLEDGEMENT

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